The arrays may be separated physically from each other or by hydrophobic surfaces. One possible way to utilize the hydrophobic strip separation is to use technology such as the Iso-Grid Microbiology System produced by QA Laboratories, Toronto, Canada.

Hydrophobic grid membrane filters (HGMF) have been in use in analytical food microbiology for about a decade where they exhibit unique attractions of extended numerical range and automated counting of colonies. One commercially-available grid is ISO-GRID™ from QA Laboratories Ltd. (Toronto, Canada) which consists of a square (60 x 60 cm) of polysulfone polymer (Gelman Tuffryn HT-450, 0.45u pore size) on which is printed a black hydrophobic ink grid consisting of 1600 (40 x 40) square cells. HGMF have previously been inoculated with bacterial suspensions by vacuum filtration and incubated on the differential or selective media of choice.

Because the microbial growth is confined to grid cells of known position and size on the membrane, the HGMF functions more like an MPN apparatus than a conventional plate or membrane filter. Peterkin *et al.* (1987) reported that these HGMFs can be used to propagate and store genomic libraries when used with a HGMF replicator. One such instrument replicates growth from each of the 1600 cells of the ISO-GRID and enables many copies of the master HGMF to be made (Peterkin *et al.*, 1987).

Sharpe *et al.* (1989) also used ISO-GRID HGMF form QA Laboratories and an automated HGMF counter (MI-100 Interpreter) and RP-100 Replicator. They reported a technique for maintaining and screening many microbial cultures.

Peterkin and colleagues later described a method for screening DNA probes using the hydrophobic grid-membrane filter (Peterkin *et al.*, 1989). These authors reported methods for effective colony hybridization directly on HGMFs. Previously, poor results had been obtained due to the low DNA binding capacity of the epoxysulfone polymer on which the HGMFs are printed. However, Peterkin *et al.* (1989) reported that the binding of DNA to the surface of the membrane was improved by treating the replicated and incubated HGMF with polyethyleneimine, a polycation, prior to contact with DNA. Although this early work uses cellular DNA attachment, and has a different objective to the present invention, the methodology described may be readily adapted for Format 3 SBH.

In order to identify useful sequences rapidly, Peterkin *et al.* (1989) used radiolabeled plasmid DNA from various clones and tested its specificity against the DNA on the

prepared HGMFs. In this way, DNA from recombinant plasmids was rapidly screened by colony hybridization against 100 organisms on HGMF replicates which can be easily and reproducibly prepared.

Manipulation with small (2-3 mm) chips, and parallel execution of thousands of the reactions. The solution of the invention is to keep the chips and the probes in the corresponding arrays. In one example, chips containing 250,000 9-mers are synthesized on a silicon wafer in the form of 8 x 8 mM plates (15 uM/oligonucleotide, Pease et al., 1994) arrayed in 8 x 12 format (96 chips) with a 1 mM groove in between. Probes are added either by multichannel pipette or pin array, one probe on one chip. To score all 4000 6-mers, 42 chip arrays have to be used, either using different ones, or by reusing one set of chip arrays several times.

In the above case, using the earlier nomenclature of the application, F=9; P=6; and F + P = 15. Chips may have probes of formula BxNn, where x is a number of specified bases B; and n is a number of non-specified bases, so that x = 4 to 10 and n = 1 to 4. To achieve more efficient hybridization, and to avoid potential influence of any support oligonucleotides, the specified bases can be surrounded by unspecified bases, thus represented by a formula such as (N)nBx(N)m.

14.2 Preparation of Support Bound Oligonucleotides

Oligonucleotides, i.e., small nucleic acid segments, may be readily prepared by, for example, directly synthesizing the oligonucleotide by chemical means, as is commonly practiced using an automated oligonucleotide synthesizer.

Support bound oligonucleotides may be prepared by any of the methods known to those of skill in the art using any suitable support such as glass, polystyrene or Teflon. One strategy is to precisely spot oligonucleotides synthesized by standard synthesizers. Immobilization can be achieved using passive adsorption (Inouye & Hondo, 1990); using UV light (Nagata *et al.*, 1985; Dahlen *et al.*, 1987; Morriey & Collins, 1989) or by covalent binding of base modified DNA (Keller *et al.*, 1988; 1989); all references being specifically incorporated herein.

Another strategy that may be employed is the use of the strong biotin-streptavidin interaction as a linker. For example, Broude *et al.* (1994) describe the use of Biotinylated probes, although these are duplex probes, that are immobilized on streptavidin-coated

magnetic beads. Streptavidin-coated beads may be purchased from Dynal, Oslo. Of course, this same linking chemistry is applicable to coating any surface with streptavidin. Biotinylated probes may be purchased from various sources, such as, e.g., Operon Technologies (Alameda, CA).

Nunc Laboratories (Naperville, IL) is also selling suitable material that could be used. Nunc Laboratories have developed a method by which DNA can be covalently bound to the microwell surface termed Covalink NH. CovaLink NH is a polystyrene surface grafted with secondary amino groups (>NH) that serve as bridge-heads for further covalent coupling. CovaLink Modules may be purchased from Nunc Laboratories. DNA molecules may be bound to CovaLink exclusively at the 5'-end by a phosphoramidate bond, allowing immobilization of more than 1 pmol of DNA (Rasmussen *et al.*, 1991).

The use of CovaLink NH strips for covalent binding of DNA molecules at the 5'-end has been described (Rasmussen et al., 1991). In this technology, a phosphoramidate bond is employed (Chu et al., 1983). This is beneficial as immobilization using only a single covalent bond is preferred. The phosphoramidate bond joins the DNA to the CovaLink NH secondary amino groups that are positioned at the end of spacer arms covalently grafted onto the polystyrene surface through a 2 nm long spacer arm. To link an oligonucleotide to CovaLink NH via an phosphoramidate bond, the oligonucleotide terminus must have a 5'-end phosphate group. It is, perhaps, even possible for biotin to be covalently bound to CovaLink and then streptavidin used to bind the probes.

More specifically, the linkage method includes dissolving DNA in water (7.5 ng/ul) and denaturing for 10 min. at 95°C and cooling on ice for 10 min. Ice-cold 0.1 M 1-methylimidazole, pH 7.0 (1-$MeIm_7$), is then added to a final concentration of 10 mM 1-$MeIm_7$. A ss DNA solution is then dispensed into CovaLink NH strips (75 ul/well) standing on ice.

Carbodiimide 0.2 M 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), dissolved in 10 mM 1-$MeIm_7$, is made fresh and 25 ul added per well. The strips are incubated for 5 hours at 50°C. After incubation the strips are washed using, e.g., Nunc-Immuno Wash; first the wells are washed 3 times, then they are soaked with washing solution for 5 min., and finally they are washed 3 times (where in the washing solution is 0.4 N NaOH, 0.25% SDS heated to 50°C).

It is contemplated that a further suitable method for use with the present invention is that described in PCT Patent Application WO 90/03382 (Southern & Maskos), incorporated herein by reference. This method of preparing an oligonucleotide bound to a support involves attaching a nucleoside 3'-reagent through the phosphate group by a covalent phosphodiester link to aliphatic hydroxyl groups carried by the support. The oligonucleotide is then synthesized on the supported nucleoside and protecting groups removed from the synthetic oligonucleotide chain under standard conditions that do not cleave the oligonucleotide from the support. Suitable reagents include nucleoside phosphoramidite and nucleoside hydrogen phosphorate.

An on-chip strategy for the preparation of DNA probe for the preparation of DNA probe arrays may be employed. For example, addressable laser-activated photodeprotection may be employed in the chemical synthesis of oligonucleotides directly on a glass surface, as described by Fodor *et al.* (1991), incorporated herein by reference. Probes may also be immobilized on nylon supports as described by Van Ness *et al.* (1991); or linked to Teflon using the method of Duncan & Cavalier (1988); all references being specifically incorporated herein.

To link an oligonucleotide to a nylon support, as described by Van Ness *et al.* (1991), requires activation of the nylon surface via alkylation and selective activation of the 5'-amine of oligonucleotides with cyanuric chloride.

One particular way to prepare support bound oligonucleotides is to utilize the light-generated synthesis described by Pease *et al.*, (1994, incorporated herein by reference). These authors used current photolithographic techniques to generate arrays of immobilized oligonucleotide probes (DNA chips). These methods, in which light is used to direct the synthesis of oligonucleotide probes in high-density, miniaturized arrays, utilize photolabile 5'-protected *N*-acyl-deoxynucleoside phosphoramidites, surface linker chemistry and versatile combinatorial synthesis strategies. A matrix of 256 spatially defined oligonucleotide probes may be generated in this manner and then used in the advantageous Format 3 sequencing, as described herein.

14.3 Preparation of Nucleic Acid Fragments

The nucleic acids to be sequenced may be obtained from any appropriate source, such as cDNAs, genomic DNA, chromosomal DNA, microdissected chromosome bands,

cosmid or YAC inserts, and RNA, including mRNA without any amplification steps. For example, Sambrook *et al.* (1989) describes three protocols for the isolation of high molecular weight DNA from mammalian cells (p. 9.14-9.23).

DNA fragments may be prepared as clones in M13, plasmid or lambda vectors and/or prepared directly from genomic DNA or cDNA by PCR or other amplification methods. Samples may be prepared or dispensed in multiwell plates. About 100-1000 ng of DNA samples may be prepared in 2-500 ml of final volume.

The nucleic acids would then be fragmented by any of the methods known to those of skill in the art including, for example, using restriction enzymes as described at 9.24-9.28 of Sambrook *et al.* (1989), shearing by ultrasound and NaOH treatment.

Low pressure shearing is also appropriate, as described by Schriefer *et al.* (1990, incorporated herein by reference). In this method, DNA samples are passed through a small French pressure cell at a variety of low to intermediate pressures. A lever device allows controlled application of low to intermediate pressures to the cell. The results of these studies indicate that low-pressure shearing is a useful alternative to sonic and enzymatic DNA fragmentation methods.

One particularly suitable way for fragmenting DNA is contemplated to be that using the two base recognition endonuclease, *Cvi*JI, described by Fitzgerald *et al.* (1992). These authors described an approach for the rapid fragmentation and fractionation of DNA into particular sizes that they contemplated to be suitable for shotgun cloning and sequencing. The present inventor envisions that this will also be particularly useful for generating random, but relatively small, fragments of DNA for use in the present sequencing technology.

The restriction endonuclease *Cvi*JI normally cleaves the recognition sequence PuGCPy between the G and C to leave blunt ends. Atypical reaction conditions, which alter the specificity of this enzyme (*Cvi*JI**), yield a quasi-random distribution of DNA fragments form the small molecule pUC19 (2688 base pairs). Fitzgerald *et al.* (1992) quantitatively evaluated the randomness of this fragmentation strategy, using a *Cvi*JI** digest of pUC19 that was size fractionated by a rapid gel filtration method and directly ligated, without end repair, to a lac Z minus M13 cloning vector. Sequence analysis of 76 clones showed that *Cvi*JI** restricts pyGCPy and PuGCPu, in addition to PuGCPy sites, and that new sequence data is accumulated at a rate consistent with random fragmentation.

As reported in the literature, advantages of this approach compared to sonication and agarose gel fractionation include: smaller amounts of DNA are required (0.2-0.5 ug instead of 2-5 ug); and fewer steps are involved (no preligation, end repair, chemical extraction, or agarose gel electrophoresis and elution are needed). These advantages are also proposed to be of use when preparing DNA for sequencing by Format 3.

Irrespective of the manner in which the nucleic acid fragments are obtained or prepared, it is important to denature the DNA to give single stranded pieces available for hybridization. This is achieved by incubating the DNA solution for 2-5 minutes at 80-90°C. The solution is then cooled quickly to 2°C to prevent renaturation of the DNA fragments before they are contacted with the chip. Phosphate groups must also be removed from genomic DNA by methods known in the art.

14.4 Preparation of DNA Arrays

Arrays may be prepared by spotting DNA samples on a support such as a nylon membrane. Spotting may be performed by using arrays of metal pins (the positions of which correspond to an array of wells in a microtiter plate) to repeated by transfer of about 20 nl of a DNA solution to a nylon membrane. By offset printing, a density of dots higher than the density of the wells is achieved. One to 25 dots may be accommodated in 1 mm^2, depending on the type of label used. By avoiding spotting in some preselected number of rows and columns, separate subsets (subarrays) may be formed. Samples in one subarray may be the same genomic segment of DNA (or the same gene) from different individuals, or may be different, overlapped genomic clones. Each of the subarrays may represent replica spotting of the same samples. In one example, a selected gene segment may be amplified from 64 patients. For each patient, the amplified gene segment may be in one 96-well plate (all 96 wells containing the same sample). A plate for each of the 64 patients is prepared. By using a 96-pin device, all samples may be spotted on one 8 x 12 cm membrane. Subarrays may contain 64 samples, one from each patient. Where the 96 subarrays are identical, the dot span may be 1 mm^2 and there may be a 1 mm space between subarrays.

Another approach is to use membranes or plates (available from NUNC, Naperville, Illinois) which may be partitioned by physical spacers e.g. a plastic grid molded over the membrane, the grid being similar to the sort of membrane applied to the bottom of

multiwell plates, or hydrophobic strips. A fixed physical spacer is not preferred for imaging by exposure to flat phosphor-storage screens or x-ray films.

14.5 Sequence Comparisons

Preferred identity and/or similarity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs including, but are not limited to, the GCG program package, including GAP (Devereux, J., et al., Nucleic Acids Research 12(1):387 (1984); Genetics Computer Group, University of Wisconsin, Madison, WI), BLASTP, BLASTN, BLASTX, and FASTA (Atschul, S.F. et al., J. Molec. Biol. 215:403-410 (1990). The BLAST X program is publicly available from the National Center for Biotechnology Information (NCBI) and other sources (BLAST Manual, Altschul, S., et al. NCB NLM NIH Bethesda, MD 20894; Altschul, S., et al., J. Mol. Biol. 215:403-410 (1990). The preferred computer program is FASTA version 3, specifically the FASTy program within the FASTA program package. Another preferred algorithm is the well known Smith Waterman algorithm which can also be used to determine identity.

Sequences can be compared to sequences in GenBank using a search algorithm developed by Applied Biosystems and incorporated into the INHERIT™ 670 Sequence Analysis System. In this algorithm, Pattern Specification Language (developed by TRW Inc., Los Angeles, CA) is used to determine regions of homology. The three parameters that determine how the sequence comparisons run are window size, window offset, and error tolerance. Using a combination of these three parameters, the DNA database can be searched for sequences containing regions of homology to the query sequence, and the appropriate sequences scored with an initial value. Subsequently, these homologous regions are examined using dot matrix homology plots to distinguish regions of homology from chance matches. Smith-Waterman alignments can be used to display the results of the homology search. Peptide and protein sequence homologies can be ascertained using the INHERIT™ 670 Sequence Analysis System in a way similar to that used in DNA sequence homologies. Pattern Specification Language and parameter windows are used to search protein databases for sequences containing regions of homology that were scored with an initial value. Dot-matrix homology plots can be examined to distinguish regions of significant homology from chance matches.

Alternatively, BLAST, which stands for Basic Local Alignment Search Tool, is used to search for local sequence alignments (Altschul SF (1993) J Mol Evol 36:290-300; Altschul, SF et al (1990) J Mol Biol 215:403-10). BLAST produces alignments of both nucleotide and amino acid sequences to determine sequence similarity. Because of the local nature of the alignments, BLAST is especially useful in determining exact matches or in identifying homologs. Whereas it is ideal for matches which do not contain gaps, it is inappropriate for performing motif-style searching. The fundamental unit of BLAST algorithm output is the High-scoring Segment Pair (HSP). An HSP consists of two sequence fragments of arbitrary but equal lengths whose alignment is locally maximal and for which the alignment score meets or exceeds a threshold or cutoff score set by the user. The BLAST approach is to look for HSPs between a query sequence and a database sequence, to evaluate the statistical significance of any matches found, and to report only those matches which satisfy the user-selected threshold of significance. The parameter E establishes the statistically significant threshold for reporting database sequence matches. E is interpreted as the upper bound of the expected frequency of chance occurrence of an HSP (or set of HSPs) within the context of the entire database search.

15. GENE THERAPY

Mutations in the polynucleotides of the invention gene may result in loss of normal function of the encoded protein. The invention thus provides gene therapy to restore normal activity of the polypeptides of the invention; or to treat disease states involving polypeptides of the invention. Delivery of a functional genes encoding polypeptides of the invention to appropriate cells is effected *ex vivo*, *in situ*, or *in vivo* by use of vectors, and more particularly viral vectors (e.g., adenovirus, adeno-associated virus, or a retrovirus), or ex vivo by use of physical DNA transfer methods (e.g., liposomes or chemical treatments). See, for example, Anderson, Nature, supplement to vol. 392, no. 6679, pp.25-20 (1998). For additional reviews of gene therapy technology see Friedmann, Science, 244: 1275-1281 (1989); Verma, Scientific American: 68-84 (1990); and Miller, Nature, 357: 455-460 (1992). Introduction of any one of the nucleotides of the present invention or a gene encoding the polypeptides of the present invention can also be accomplished with extrachromosomal substrates (transient expression) or artificial chromosomes (stable expression). Cells may also be cultured *ex vivo* in the presence of proteins of the present

invention in order to proliferate or to produce a desired effect on or activity in such cells. Treated cells can then be introduced in vivo for therapeutic purposes. Alternatively, it is contemplated that in other human disease states, preventing the expression of or inhibiting the activity of polypeptides of the invention will be useful in treating the disease states. It is contemplated that antisense therapy or gene therapy could be applied to negatively regulate the expression of polypeptides of the invention.

Other methods inhibiting expression of a protein include the introduction of antisense molecules to the nucleic acids of the present invention, their complements, or their translated RNA sequences, by methods known in the art, the removal of the nucleic acids of the present invention such as using targeted deletion methods, or the insertion of a negative regulatory element such as a silencer, which is tissue specific. Further, the polypeptides of the present invention can be inhibited by the introduction of antisense molecules that hybridize to nucleic acids that encode for the polypeptides of the present invention and by the removal of a gene that encode for the polypeptides of the present invention.

The present invention still further provides cells genetically engineered *in vivo* to express the polynucleotides of the invention, wherein such polynucleotides are in operative association with a regulatory sequence heterologous to the host cell which drives expression of the polynucleotides in the cell. These methods can be used to increase or decrease the expression of the polynucleotides of the present invention.

Knowledge of DNA sequences provided by the invention allows for modification of cells to permit, increase, or decrease, expression of endogenous polypeptide. Cells can be modified (e.g., by homologous recombination) to provide increased polypeptide expression by replacing, in whole or in part, the naturally occurring promoter with all or part of a heterologous promoter so that the cells express the protein at higher levels. The heterologous promoter is inserted in such a manner that it is operatively linked to the desired protein encoding sequences. See, for example, PCT International Publication No. WO 94/12650, PCT International Publication No. WO 92/20808, and PCT International Publication No. WO 91/09955. It is also contemplated that, in addition to heterologous promoter DNA, amplifiable marker DNA (e.g., ada, dhfr, and the multifunctional CAD gene which encodes carbamyl phosphate synthase, aspartate transcarbamylase, and dihydroorotase) and/or intron DNA may be inserted along with the heterologous promoter DNA. If linked to the desired protein coding sequence, amplification of the marker DNA by

standard selection methods results in co-amplification of the desired protein coding sequences in the cells.

In another embodiment of the present invention, cells and tissues may be engineered to express an endogenous gene comprising the polynucleotides of the invention under the control of inducible regulatory elements, in which case the regulatory sequences of the endogenous gene may be replaced by homologous recombination. As described herein, gene targeting can be used to replace a gene's existing regulatory region with a regulatory sequence isolated from a different gene or a novel regulatory sequence synthesized by genetic engineering methods. Such regulatory sequences may be comprised of promoters, enhancers, scaffold-attachment regions, negative regulatory elements, transcriptional initiation sites, regulatory protein binding sites or combinations of said sequences. Alternatively, sequences which affect the structure or stability of the RNA or protein produced may be replaced, removed, added, or otherwise modified by targeting. These sequence include polyadenylation signals, mRNA stability elements, splice sites, leader sequences for enhancing or modifying transport or secretion properties of the protein, or other sequences which alter or improve the function or stability of protein or RNA molecules.

The targeting event may be a simple insertion of the regulatory sequence, placing the gene under the control of the new regulatory sequence, *e.g.*. inserting a new promoter or enhancer or both upstream of a gene. Alternatively, the targeting event may be a simple deletion of a regulatory element, such as the deletion of a tissue-specific negative regulatory element. Alternatively, the targeting event may replace an existing element; for example, a tissue-specific enhancer can be replaced by an enhancer that has broader or different cell-type specificity than the naturally occurring elements. Here, the naturally occurring sequences are deleted and new sequences are added. In all cases, the identification of the targeting event may be facilitated by the use of one or more selectable marker genes that are contiguous with the targeting DNA, allowing for the selection of cells in which the exogenous DNA has integrated into the cell genome. The identification of the targeting event may also be facilitated by the use of one or more marker genes exhibiting the property of negative selection, such that the negatively selectable marker is linked to the exogenous DNA, but configured such that the negatively selectable marker flanks the targeting sequence, and such that a correct homologous recombination event with sequences in the

host cell genome does not result in the stable integration of the negatively selectable marker. Markers useful for this purpose include the Herpes Simplex Virus thymidine kinase (TK) gene or the bacterial xanthine-guanine phosphoribosyl-transferase (gpt) gene.

The gene targeting or gene activation techniques which can be used in accordance with this aspect of the invention are more particularly described in U.S. Patent No. 5,272,071 to Chappel; U.S. Patent No. 5,578,461 to Sherwin et al.; International Application No. PCT/US92/09627 (WO93/09222) by Selden et al.; and International Application No. PCT/US90/06436 (WO91/06667) by Skoultchi et al., each of which is incorporated by reference herein in its entirety.

16. TRANSGENIC ANIMALS

In preferred methods to determine biological functions of the polypeptides of the invention in vivo, one or more genes provided by the invention are either over expressed or inactivated in the germ line of animals using homologous recombination [Capecchi, Science 244:1288-1292 (1989)]. Animals in which the gene is over expressed, under the regulatory control of exogenous or endogenous promoter elements, are known as transgenic animals. Animals in which an endogenous gene has been inactivated by homologous recombination are referred to as "knockout" animals. Knockout animals, preferably non-human mammals, can be prepared as described in U.S. Patent No. 5,557,032, incorporated herein by reference. Transgenic animals are useful to determine the roles polypeptides of the invention play in biological processes, and preferably in disease states. Transgenic animals are useful as model systems to identify compounds that modulate lipid metabolism. Transgenic animals, preferably non-human mammals, are produced using methods as described in U.S. Patent No 5,489,743 and PCT Publication No. WO94/28122, incorporated herein by reference.

Transgenic animals can be prepared wherein all or part of a polynucleotides of the invention promoter is either activated or inactivated to alter the level of expression of the polypeptides of the invention. Inactivation can be carried out using homologous recombination methods described above. Activation can be achieved by supplementing or even replacing the homologous promoter to provid for increased protein expression. The homologous promoter can be supplemented by insertion of one or more heterologous enhancer elements known to confer promoter activation in a particular tissue.

The present invention is illustrated in the following examples. Upon consideration of the present disclosure, one of skill in the art will appreciate that many other embodiments and variations may be made in the scope of the present invention. Accordingly, it is intended that the broader aspects of the present invention not be limited to the disclosure of the following examples. Example 1 addresses cloning of IL-1Hy2 cDNA, Example 2 addresses identification of polymorphisms, Example 3 addresses tissue expression of IL-1Hy2 mRNA and polypeptide, Example 4 addresses chromosomal localization of IL-1Hy2 DNA, Example 5 addresses identification of an IL-1 receptor binding region and binding to IL-1 receptor, Example 6 addresses IL-1Hy2 polypeptide expression in *E. coli*, Example 7 addresses confirmation of IL-1Hy2 biological activities through assessment of its modulating effect on IL-1 related activities and IL-1 related disorders, Example 8 addresses the sequencing of the IL-1Hy2 human genomic BAC clone, Example 9 addresses the sequencing of IL-1 Hy2 mouse genomic BAC clone, Example 10 addresses inhibition of IL-1β induced IL-6 production by IL-1 Hy2, Example 11 addresses the inhibition of IL-18 activity by IL-1 Hy2, Example 12 addresses IL-1 Hy2 binding to the IL-1 receptor, Example 13 addresses expression of IL-1 Hy2 in mammalian cells

EXAMPLE 1

Cloning of IL-1 Hy2 cDNA

A plurality of novel nucleic acids were obtained from the FSK001 cDNA library (prepared from human fetal skin tissue mRNA purchased from Invitrogen, San Diego, CA) using standard PCR, SBH sequence signature analysis and Sanger sequencing techniques. The inserts of the library were amplified with PCR using primers specific for pSport1 (GIBCO BRL, Grand Island, N.Y) vector sequences which flank the inserts. These samples were spotted onto nylon membranes and interrogated with oligonucleotide probes to give sequence signatures. The clones were clustered into groups of similar or identical sequences, and single representative clones were selected from each group for gel sequencing. The 5' sequence of the amplified inserts was then deduced using the reverse M13 sequencing primer in a typical Sanger sequencing protocol. PCR products were purified and subjected to flourescent dye terminator cycle sequencing. Single pass gel sequencing was done using a 377 Applied Biosystems (ABI) sequencer. One cDNA insert was identified by sequencing of several hundred base pairs (approximately 1-386 of SEQ ID

NO: 1) as a novel sequence related to IL-1Ra that had not been previously reported in public databases. The remaining sequence of SEQ ID NO: 1 was obtained by further sequencing of the entire cDNA insert of the same clone; the sequence was confirmed in part by sequencing of 5' RACE PCR products from fetal skin and adult brain cDNA libraries. This sequence and the clone were designated by code name CG149 and clone name RTA00003379F.h.20 (later redesignated pIL-1Hy2 and deposited at the ATCC on May 21, 1999 under Accession No. PTA-96), and the encoded polypeptide was designated IL-1Ra-Hy2 (later redesignated IL-1Hy2).

EXAMPLE 2

Identification of polymorphisms

Sequencing of a number of PCR products from various cDNA libraries revealed several potential polymorphisms, which are described with reference to the nucleotide sequence numbering of SEQ ID NO: 1.

At nucleotide 125 of SEQ ID NO: 1, the "T" may be replaced with a "C", resulting in a codon change from "GAT" to "GAC" (a silent mutation, as both codons encode the amino acid Asp). At nucleotide 184 of SEQ ID NO: 1, the "C" may be replaced with a "T", resulting in a codon change from "ACA" (encoding Thr) to "ATA" (encoding Ile). At nucleotide 205 of SEQ ID NO: 1, the "A" may be replaced with a "C", resulting in a codon change from "GAC" (Asp) to "GCC" (Ala). The changes in the amino acid sequence may be reflected in differences in the biological activities of the molecules, which can be confirmed by testing in any of the activity assays described herein.

EXAMPLE 3

Tissue Expression Study

3.1 In situ Hybridization

Gene expression of human IL-1 Hy2 was analyzed using a semi-quantitative PCR-based technique. A panel of cDNA libraries derived from human tissue (from Clontech and Invitrogen) was screened with IL-1Hy2 specific primers [5'-CCGCACCAAGGTCCCCATTTTC-3' (nucleotides 206-227) , SEQ ID NO: 10 and 3'-GAGCCCACAAGGATAACCCAGG-5' (nucleotides 728-707), SEQ ID NO: 11] to examine the mRNA expression of IL-1Hy2 in the following human tissues and cell types:

heart, kidney, lung, placenta, liver, ovary, lymph node, spleen, testes, thymus, fetal liver, fetal skin, fetal spleen and macrophage. PCR assays (94 °C for 30 sec., 58 °C for 30 sec., 72 °C for 30 sec., for 30 cycles) were performed with 20 ng of cDNA derived from human tissues and cell lines and 10 picomoles of the IL-1Hy2 gene-specific primers. The 522 bp PCR product was identified through gel electrophoresis. Amplified products were separated on an agarose gel, transfered and chemically linked to a nylon filter. The filter was then hybridized with a radioactively labeled (^{33}Palpha-dCTP) double-stranded probe generated from the full-length SEQ ID NO: 1 sequence using a Klenow polymerase, random prime method. The filters were washed (high stringency) and used to expose a phosphorimaging screen for several hours. Bands indicated the presence of cDNA including SEQ ID NO: 1 sequences in a specific library, and thus mRNA expression in the corresponding cell type or tissue.

IL-1Hy2 mRNA was observed to be expressed in kidney, spleen, and fetal skin. Similar to IL-1Hy2, IL-1Ra and IL-1Hy1 mRNA are also expressed in the human fetal skin tissues, suggesting that this family of proteins may share some physiologic functions.

Additional studies were performed to localize IL-1 Hy2 mRNA expression as described by D'Andrea et al. (J. Sur. Path, 1: 191-203,1995). IL-1 Hy2 mRNA was detected in serial sections of human normal tonsil and kidney by DIG-labeled probes consisting of nucleotide 396 to 568 of SEQ ID NO: 14. The slides were hybridized with the IL-iHy2 probes for 2 hours at 54°C. Subsequently, the slides were washed with 2x SSC at room temperature and then washed with 0.1x SSC at 54°C. After the stringency rinses, 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (BCIP/NBT) was used a chromagen. For visual detection, the slides were counter-stained with Eosin and examined under a light microscope.

3.2 Immunohistochemistry

The serial sections of normal tonsil were also stained with polyclonal antibodies specific for IL-1 Hy2 prepared by immunizing rabbits with IL-1 Hy2 peptide: 43-56 of SEQ. ID NO.: 2 using conventional methods [see, e.g. Harlow et al., "Antibodies: A Laboratory Manual". Cold Spring Harbor Laboratories, Cold Spring Harbor, NY (1998)] and control preimmune serum form the immunized rabbits. In addition, the slides were stained with antibodies for CD20, Ki67, CD3, CD1a, CD14, CD68 and LN5. Antibody binding was

detected with biotinylated secondary antibodies and streptavidin-HRP . AEC Haishen was used as the chromagen for detection and the slides were counter-stained with hematoxylin. IL-1 Hy2 expression was visually detected under a light microscope.

IL-1 Hy2 mRNA was detected it the distal tubules of the kidney, the glomeruli of the kidney, the Bowman's capsule epithelia, capillary epithelia, and a subset of white blood cells within the blood vessels. In the tonsil, IL-1 Hy2 mRNA and protein were detected in a subset of B-cells (CD20 positive) in the germinal center, most of which were proliferating according to Ki67 staining. IL-1 Hy2 was also expressed in the basal squamous epithelial of the skin surrounding the tonsil.

EXAMPLE 4

Chromosomal Localization Study

Chromosome mapping technologies allow investigators to link genes to specific regions of chromosomes. Chromosomal mapping was performed using the NIGMS human/rodent somatic cell hybrid mapping panel as described by Drwinga, H. L. et al., Genomics, 16, 311- 314, 1993 (human/rodent somatic cell hybrid mapping panel #2 purchased from the Coriell Institute for Medical Research, Camden, New Jersey). 60 ng of DNA from each sample in the panel was used as template, and 10 picomoles of the same IL-1Hy2 gene-specific oligonucleotides used in Example 3 were used as primers in a PCR assay (94 °C for 30 sec., 58 °C for 30 sec., 72 °C for 30 sec., for 30 cycles). PCR products were analyzed by gel electrophoresis. The 800 bp genomic PCR product was detected only in the human/rodent somatic cell hybrid DNA containing human chromosome 2.

Gene family members are often linked to specific regions of chromosomes owing to intrachromosomal gene duplication events that give rise to multimember gene families during the process of evolution. The interleukin-1 gene family has been mapped to chromosome 2. More specifically, all of the interleukin 1 genes (IL-1α, IL-1β) and the receptors (IL-1 RI and IL-1 RII), as well as the receptor antagonist IL-1ra and the newly identified IL-1 Hy2 have been found to be situated in chromosome 2. The identification of IL-1 Hy2 sequences in this same region establishes its physical linkage to the interleukin-1 locus which indicates that IL-1 Hy2 functions as a modulator of the inflammatory response.

EXAMPLE 5

Interleukin-1 Receptor Binding Domain and Interleukin-1 Receptor Assay

The receptor binding region of both IL-1β and IL-1 Ra have been mapped to an 18 amino acid region in the carboxy terminal half of the proteins (i.e., residues 88-105 of IL-1β) by site-directed mutagenesis and protein modification studies.

IL-1 Hy2 and fragments thereof that include a receptor binding region are useful as reagents to identify cells and tissues expressing IL-1 receptors. The IL-1 receptor binding assay described in Hannum et al. Nature 343:336-340 (1990) may be used. Briefly, highly radioactive recombinant SEQ ID NOS: 2, 4 or 13 is prepared by growing E.coli expressing either of SEQ ID NOS: 2 ,4 or 13 on M9 medium containing [^{35}S] sulphate and purifying the labeled recombinant polypeptide by chromatography on a Mono-S column. The labeled polypeptide is incubated with the cells or tissue under standard IL-1 binding assay conditions, and [^{35}S] binding. Significant [^{35}S] binding indicates the presence of IL-1 receptors.

EXAMPLE 6

Expression of IL-1 Hy2 in E. coli

SEQ ID NOS: 1, 12 or 14 are expressed in E. coli by subcloning the entire coding region into a prokaryotic expression vector. The expression vector (pQE16) used is from the QIAexpression prokaryotic protein expression system (Qiagen). The features of this vector that make it useful for protein expression include: an efficient promoter (phage T5) to drive transcription; expression control provided by the lac operator system, which can be induced by addition of IPTG (isopropyl-β-D-thiogalactopyranoside), and an encoded His_6 tag. The latter is a stretch of 6 histidine amino acid residues which can bind very tightly to a nickel atom. The vector can be used to express a recombinant protein with a His_6 tag fused to its carboxyl terminus, allowing rapid and efficient purification using Ni-coupled affinity columns.

PCR is used to amplify the coding region which is then ligated into digested pQE16 vector. The ligation product is transformed by electroporation into electrocompetent E.coli cells (strain M15[pREP4] from Qiagen), and the transformed cells are plated on ampicillin-containing plates. Colonies are screened for the correct insert in the proper orientation using a PCR reaction employing a gene-specific primer and a vector-specific

primer. Positives are then sequenced to ensure correct orientation and sequence. To express IL-1 Hy2, a colony containing a correct recombinant clone is inoculated into L-Broth containing 100 μg/ml of ampicillin, 25 μg/ml of kanamycin, and the culture was allowed to grow overnight at 37°C. The saturated culture is then diluted 20-fold in the same medium and allowed to grow to an optical density at 600 nm of 0.5. At this point, IPTG is added to a final concentration of 1 mM to induce protein expression. The culture is allowed to grow for 5 more hours, and then the cells are harvested by centrifugation at 3000xg for 15 minutes.

The resultant pellet is lysed using a mild, nonionic detergent in 20mM Tris HCl (pH 7.5) (B-PER™ Reagent from Pierce), or by sonication until the turbid cell suspension turned translucent. The lysate obtained is further purified using a nickel containing column (Ni-NTA spin column from Qiagen) under non-denaturing conditions. Briefly, the lysate is brought up to 300mM NaCl and 10mM imidazole and centrifuged at 700xg through the spin column to allow the His-tagged recombinant protein to bind to the nickel column. The column is then washed twice with Wash Buffer (50mM NaH_2PO_4, pH8.0; 300mM NaCl; 20mM imidazole) and is eluted with Elution Buffer (50mM NaH_2PO_4, pH8.0; 300mM NaCl; 250mM imidazole). All the above procedures are performed at 4°C. The presence of a purified protein of the predicted size is confirmed with SDS-PAGE.

EXAMPLE 7

Evaluation of IL-1 Hy2 Activities *In Vitro and In Vivo*

7.1 Binding to the Interleukin-1 Receptor

A cell binding assay is carried out to demonstrate that IL-1 Hy2 binds to the Interleukin-1 receptor. Briefly, cell binding of the recombinant protein with and without the presence of 100-fold greater amounts of non tagged Interleukin-1 ßeta (IL-1ß) ligand is analyzed by using fluorescent antibodies specific for a IL-1 Hy2 polypeptide (e.g. specific for an express tag within the recombinant polypeptide) on the fluorescent activated cell sorter (FACS). In each reaction, 10^6 cells NHDF (normal human dermal fibroblasts) are resuspended in 100 μl of FACS buffer (distilled PBS and 3% calf serum and 0.01% azide). Cell binding is done by adding 5 nM recombinant IL-1 Hy2 in 100 μl cell suspension and as a competition in one reaction, 500 nM of recombinant IL-1 ß is also added. The cells are incubated on ice for 1 hr. The cells are pelleted, 200 μl of 0.2 mM BS3 (crosslinker) is

added, and the cells are kept on ice for 30 min. Next, 10 ul 1 M Tris pH 7.5 is added and the cells are incubated for 15 minutes on ice. The cells are pelleted, washed 1 time in FACS buffer, resuspended in 100 µl volume of FACS buffer and 2 µl primary antibody (anti-express tag antibody 1mg/ml) is added, and incubated on ice for 30 minutes. The cells are pelleted, washed with FACS buffer, and resuspended in FACS buffer (100 ul volume). The secondary antibody (phycoerythrin conjugated) 2ul of anti-mouse Ig (1mg/ml) is added and the cells are incubated for 30 minutes on ice. The cells are again pelleted, washed two times with FACS buffer, resuspended in 0.5 ml FACS buffer and analyzed on FACS. A shift in the fluorescence is expected to be observed in the cells treated with the recombinant tagged IL-1 Hy2. This binding is shown to be specific if it is competed off with the non tagged IL-1 ß protein. The results will indicate binding of IL-1 Hy2 to the IL-1 receptor.

7.2 IL-1 Antagonist Activity

IL-1 antagonist activity is determined using a prostaglandin E2 (PGE_2) based assay as follows. Cells are plated at 20,000 cells per well in a 96 well plate 24 hours before the assay. The cells are then treated with 25 pg/ml recombinant human IL-1β for 7 hours. To evaluate inhibition of IL-1β stimulated PGE_2 release by IL-1Hy2 in comparison to IL-1Ra, the cells are pretreated with various amounts of IL-1Hy2 or IL-1Ra for two hours before the addition of IL-1β. The supernatants are then collected and cell debris is removed by centrifugation. The amounts of PGE_2 in the supernatants are determined by ELISA using the PGE_2 assay system (R&D Systems) according to the manufacturer's protocol.

This assay was carried out with IL-1 Hy2 as follows. To stimulate IL-1β induced PGE_2 production, human fibroblasts (CCD 1098; accession no. CRL 2127) were plated at 20,000 cells per well in a 96 well plate 24 hours before the assay. The cells were then washed once with fresh media and incubated for 16 hours with fresh media containing 1 ng/ml recombinant human IL-1β. To evaluate inhibition of IL-1β stimulated PGE_2 release by IL-1 Hy2 in comparison to IL-1Ra, the cells were treated with various concentrations of IL-2 Hy2 or IL-1Ra together with IL-1β. After the 16 hour stimulation at 37°C in a 5%CO_2 incubator, the culture plates were centrifuged for 5 minutes at 4000 rpm to remove cellular debris. The amounts of PGE_2 were determined by assaying 100 µl of supernatant with the PGE_2 ELISA assay kit (R & D Systems) according to the manufacturer's protocol.

The addition of IL-1 Hy2 to the IL-1β stimulated cultures resulted in a dose-dependent partial decrease in PGE_2 production. At a concentration of 1000 fold excess, IL-1 Hy2 inhibited IL-1β induced PGE_2 production 40-60%. As a control and a means for comparison, IL-1Ra completely inhibited PGE_2 production at a concentration of 100 fold excess. The fact that IL-1 Hy2 only partially inhibits IL-1β activity may be beneficial in the treatment of inflammatory disease states due to fewer side effects. It is possible that more highly purified preparations of IL-1 Hy1 may show complete inhibition in this assay.

7.3 Inhibition Of Interleukin-1 Induced Cell Proliferation

Murine D10 T cells are obtained from the American Type Culture Collection (Rockville, Md.). Cells are maintained in Dulbecco's modified Eagle medium and Ham's F-12 medium (1:1) containing 10 mM HEPES buffer (pH 7.4) and 10% fetal bovine serum. All tissue culture reagents contained less than 0.25 ng/mL endotoxin as measured by the limulus amebocyte assay.

Murine D10 cells, an Interleukin-1 dependent T-cell line, are used to measure Interleukin-1 mitogenic activity. Cell proliferation in the present of Interleukin-1 with and without the IL-1 Hy2 polypeptides of the invention is assessed by incorporation of (sup 3 H) thymidine as previously described (Bakouche, O., et al. J. Immunol. 138:4249-4255, 1987). In a preferred embodiment, antagonists and agonist of the IL-1 Hy2 polypeptides of the invention are identified in this assay by adding the candidate compounds with the Interleukin-1 and IL-1 Hy2 polypeptides of the invention and measuring the change in cell proliferation caused by the candidate compound.

7.4 Inhibition Of Interleukin-1 Induced Cell Cytotoxicity

Inhibition of Interleukin-1-induced cytotoxicity is studied using an appropirate cell line, such as, for example, A375 tumor cells plated at a density of 6000 cells per well in 96-well microliter plates. After overnight attachment, Interleukin-1 (3-300 ng/mL) is added in the presence or absence of NAA or NMA. After cells are incubated for 3 days, (sup 3 H) thymidine is added (1 mu Ci per well) for an additional 2 hours. Cells are harvested onto glass fiber disks (PHD Cell Harvested; Cambridge Technology, Inc., Watertown, Ma.) Disks are air dried overnight, and radioactivity is determined with a Model 1900TR Scintillation Counter (Packard Instrument Division, Downers Grove, Ill.)

7.5 Induction Of Nitrite Synthesis In Smooth Muscle Cells

Aortic smooth muscle cells are cultured by explanting segments of the medial layer of aortas from adult male Fischer 344 rats. Aortas are removed aseptically and freed of adventitial and endothelial cells by scraping both the luminal and abluminal surfaces. Medial fragments are allowed to attach to Primaria 25-cm sup 2 tissue culture flasks (Becton-Dickinson, Lincoln Park, N.J.) which are kept moist with growth medium until cells emerged. Cultures are fed twice weekly with medium 199 containing 10% fetal bovine serum, 25 mM HEPES buffer (pH 7.4), 2mM L-glutamine, 40 mu g/mL endothelial cell growth supplement (Biomedical Technologies, Inc., Stoughton, Mass.) and 10 mu g/ml gentamicin (GIBCO BRL, Grand Island, N.Y.). When primary cultures become confluent, they are passaged by trypsinization, and explants are discarded. For these studies, cells from passages 12-14 are seeded at 20,000 per well in 96-well plates and are used at confluence (60,000-80,000 cells per well). The cells exhibit the classic smooth muscle cell phenotype with hill and valley morphology, and they stain positively for smooth muscle actin.

Rat aortic smooth muscle cells are incubated with RPMI-1640 medium containing 10% bovine calf serum, 25 mM HEPES buffer 7.4), 2 mM glutamine, 80 U/mL penicillin, 80 mu g/mL streptomycin, 2 mu g/mL fungizone, and Interleukin-1, IFN-gamma, and various inhibitors. At the desired times, nitrite concentration in the culture medium is measured using the standard Griess assay (Green, L., et al. Anal. Biochem. 126:131-138, 1982) adapted to a 96-well microtiter plate reader (Gross, S. S., et al. Biochem. Biophys. Res. Commun. 178:823-829, 1991). Thus, 100 muL of Griess reagent (0.5% sulfanilic acid, 0.05% naphthalenediamine, and 2.5% phosphoric acid) is added to an equal volume of culture medium, and the OD sub 550 is measured and related to nitrite concentration by reference to a standard curve. The background OD sub 550 of medium incubated in the absence of cells is subtracted from experimental values.

Rat aortic smooth muscle cells are incubated with RPMI-1640 medium containing 10% bovine calf serum, 25 mM HEPES buffer (pH 7.4), 2 mM glutamine, 80 mu g/mL penicillin, 80 mu g/mL steptomycin, 2 mu g/mL fungizone, 30 mu g/mL lipopolysaccharide (Escherichia coli 0111:B4), and 50 U/mL IFN-y. Cells are harvested after 24 hours, and cytosol is prepared (Gross, S. S., et al. Biochem. Biophys. Res. Commun. 178:823-829, 1991). Cytosolic NO synthase activity is assayed by the Fe sup 2+ -myoglobin method

described previously (Gross, S. S., et al. Biochem. Biophys. Res. Commun. 178:823-829, 1991).

7.6 Alloreactivity Determined By Lymph Node Weight Gain

Experiments are conducted to show that systemic administration of the IL-1 Hy2 polypeptides of the invention suppress a localized, T cell-dependent, immune response to alloantigen presented by allogeneic cells. Mice are injected in the footpad with irradiated, allogeneic spleen cells. The mice are then injected in the contralateral footpad with irradiated, syngeneic spleen cells. An alloreactive response (marked by proliferation of lymphocytes and inflammation) occurs in the footpad receiving the allogeneic cells, which can be measured by determining the increase in size and weight of the popliteal lymph node draining the site of antigen deposition relative to controls or by an increase in cellularity.

Specific pathogen free 8-12 week old BALB/c (H-2 sup d) and C57BL/6 (H-2 sup b) mice (Jackson Laboratory, Bar Harbor, Me.) are used in this experiment. 48 BALB/c mice are divided into 16 groups, each having 3 mice (unless otherwise indicated). Each group of mice received a different mode of treatment. On day 0 the left footpads of all mice are injected intracutaneously with 107 irradiated (2500R), allogeneic spleen cells from C57BL/6 mice in 50 ul of RPMI-1640 (Gibco) as antigen and the right contralateral footpads of the same mice are injected with 10 sup 7 irradiated (2500R), syngeneic spleen cells from BALB/c mice.

Seven days after antigen administration, the mice are sacrificed and the popliteal lymph nodes (PLN) are removed from the right and left popliteal fossa by surgical dissection. Lymph nodes are weighed and the results expressed as the difference (DELTA) in weight (mg) of the lymph node draining the site of allogeneic cell injection and the weight of the node draining the syngeneic cell injection site. Lymph nodes draining the syngeneic cell injection site weighed approximately 1 mg, regardless of whether they are obtained from mice treated with MSA or IL-1 Hy2 polypeptides of the invention, and did not differ significantly in weight from nodes obtained from mice given no cell injection.

7.7 Suppression Of Organ Graft Rejection In Vivo

Neonatal C57BL/6 (H-2 sup b) hearts are transplanted into the ear pinnae of adult BALB/c (H-2 sup d) recipients utilizing the method of Fulmer et al., Am. J. Anat. 113:273,

1963, modified as described by Trager et al., Transplantation 47:587, 1989, and Van Buren et al., Transplant. Proc. 15:2967, 1983. Survival of the transplanted hearts is assessed by visually inspecting the grafts for pulsatile activity. Pulsatile activity is determined by examining the ear-heart grafts of anesthetized recipients under a dissecting microscope with soft reflected light beginning on day 5 or 6 post transplant. The time of graft rejection is defined as the day after transplantation on which contractile activity ceases.

Recipient mice are transplanted on day 0 and injected with either IL-1 Hy2 polypeptides of the invention plus MSA (mouse serum albumin, 100 ng) or with MSA alone on days 0 through 6, alternating i.p. and s.c. routes. In a second heart transplant experiment, the mice are injected with MSA alone on days 0 through 2, i.p. route only.

7.8 Suppression Of Inflammatory Arthritis

20 rats are divided into 4 groups, designated Groups G-J, each having 5 rats. All rats are immunized by subcutaneous injection. On day 21 following immunization with mBSA, an inflammatory arthritis response is elicited. On the same day, a negative control group is injected with a 0.2 ml volume of saline. Groups are injected with increasing amounts of IL-1 Hy2 polypeptides of the invention. Interleukin-1 is injected in one group as a positive control. The diameter of the largest egion of the treated joints is measured using a caliper on days 2, 4, 6 and 8 relative to day 0 intra-articular injection of antigen.

7.9 Activity in a Pancreatitis Model

Acute edematous, necrotizing pancreatitis is induced in adult male Swiss mice weighing more than 35 grams using caerulein--an analog of cholecystokinin. Mice are divided into four groups with three of the groups receiving caerulein 50 mu g/kg by intraperitoneal (IP) injection in four doses over three hours as previously described. (Murayama et al., Arch Surg 1990;125:1570-1572; Tani et al., International J Pancreatology 1987;2:337-348; Schoenberg et al., Free Radical Biology & Medicine 1992;12:515-522; Heath et al., Pancreas 1993;66:41-45; Saluja et al., Amer Physiological Society 1985: G702-G710; Manso et al., Digestive Disease and Sciences 1992;37:364-368). Group 1 is a control group (n-9) which receives only IP saline injections. Group 2 (n=12) is an untreated disease control. Group 3 (n=12) receives three injections of drug (10 mg/kg/hr)

starting one hour prior to induction of pancreatitis. Group 4 (n=12) receives three injections of drug (10 mg/kg/hr) starting one hour after induction of pancreatitis.

After a suitable time period, all animals are euthanized, the blood collected, and the pancreata surgically excised and weighed. Serum is assayed for amylase, lipase, IL-6, and TNF levels. Each pancreas is fixed, stained, and graded histologically in a blinded fashion or interstitial edema, granulocyte infiltration, acinar vacuolization, and acinar cell. Additionally, serum levels of IL-1 Hy2 are determined, therefore allowing comparisons between dosage, serum level, systemic cytokine response, and degree of pancreatic damage.

Interleukin-6, Interleukin-1, Interleukin-1 receptor antagonist, and TNF are measured by commercially available ELISA kits (Genzyme Corp., Boston, Mass.). All specimens are run in triplicate. Serum levels of amylase and lipase are measured on a Kodak Ectachem 700 automated analyzer (Eastman Kodak Company, Rochester, N.Y.).

Histologic slides are prepared as is known in the art after rapid excision and subsequent fixation in 10% formalin. The tissues are paraffin embedded as is known in the art and then stained with Hematoxylin and Eosin in a standard fashion. These slides are examined and graded in a blinded fashion by a board certified pathologist.

EXAMPLE 8

Sequencing of IL-1 Hy2 Human Genomic BAC Clone

To understand the genomic organization of the IL-1 Hy2 gene, a commercial human BAC library (Research Genetics)was screened with the full length IL-1 Hy2 cDNA using standard procedures. The BAC clone containing the human genomic IL-1 Hy2 gene was sequenced by conventional methods and is set forth as SEQ ID NO: 15. Based on the sequences, exons of the IL-1 Hy2 gene were predicted using the GenScan software (Stanford University). This analysis indicated that the IL-1 Hy2 cDNA should contain additional sequences at the 5′ end in addition to those set forth in SEQ ID NO: 1.

The predicted cDNA sequence based on the genomic DNA sequence encoding IL-1 Hy2 was compared to the corresponding cDNA sequence. This analysis indicated that the predicted cDNA sequence based on the human genomic sequence of IL-1 Hy2 (SEQ ID NO: 12) contains a thymidine (T) at nucleotide 279 (see Figure 2), while the IL-1 Hy2 cDNA sequence (SEQ ID NO: 14; Figure 4) contains a cytosine (C) at position 279. The change in nucleotides (C→T) would extend the IL-1 Hy2 open reading frame in the 5′

direction, resulting in a 200 amino acid polypeptide, while the cDNA sequence (SEQ ID NO: 14; Figure 4) encodes a 152 amino acid polypeptide (SEQ ID NO: 2).

The predicted cDNA sequence (SEQ ID NO: 12) is 1366 nucleotides which contains an open reading frame (nucleotides 278 to 880) that encodes a predicted polypeptide of 200 amino acids (SEQ ID NO: 13; Figure 2). However, the sequences surrounding the translation initiation codon at nucleotide 422 of SEQ ID NO: 12 are more similar to the Kozak translation start site consensus than the sequences surrounding nucleotide 278. Therefore, it is also possible that the IL-1 Hy2 polypeptide is encoded by a shorter open reading frame between nucleotides 422 and 880 of SEQ ID NO: 12, which encodes a predicted polypeptide of 152 amino acid (SEQ ID NO: 2).

EXAMPLE 9

Sequencing of IL-1 Hy2 Mouse Genomic BAC Clone

A commercial mouse BAC library (Research Genetics)was screened with the full length IL-1 Hy2 cDNA using standard procedures. The BAC clone containing the mouse IL-1 Hy2 gene was sequenced by conventional methods and is set forth as SEQ ID NO: 17. Based on the sequences, exons of the mouse IL-1 Hy2 gene were predicted using the GenScan software (Stanford University). This analysis indicated that the mouse IL-1Hy2 gene contains 4 exons. The predicted cDNA encoding the mouse IL-1 Hy protein is set forth as SEQ ID NO: 16. The murine IL-1 Hy2 polypeptide translation initiates at nucleotide 1 and terminates at nucleotide 457 of SEQ ID NO: 17. The mouse and human IL-1 Hy2 polypeptide sequences share 81.7% homology. The murine genomic DNA sequence can be used to generate transgenic animals which overexpress the IL-1 Hy2 polypeptide or have the IL-1 Hy2 gene knocked out as described above in Section 16.

EXAMPLE 10

Inhibition of IL-1β Induced IL-6 Production

Inhibition of Interleukin-1β induced IL-6 production was studied using human endothelial cells from umbilical vein (Huvec). Huvec cells were seeded at 2 X 10^4 cells per well in a 96-well plate the day before cell stimulation. On the day of stimulation, cells were washed once with fresh medium (F12 medium with 100 μg/ml heparin, 50 μg/ml endothelial growth supplement and 10% fetal bovine serum) and replated with 200 μl of

fresh medium [without supplements] in each well. The Huvec cells were then stimulated with 100 pg/ml (final volume) of IL-1β. Although this assay was done with IL-1β, any cytokine of interest can be used. To test IL-6 inhibition, different concentrations of IL-1Hy2 (ranging from 10x to 1000x the concentration of IL-1β) or IL-1ra (ranging from 10x to 1000x IL-1β concentration) were added to the wells with the IL-1β.

After 16 hours of cell stimulation, the culture plate was spun for five minutes at 4000 rpm to remove cell debris. To test for the presence of IL-6, 100 µl of supernatant was removed and assayed with a human IL-6 immunoassay kit (R&D Systems) according to the manufacturer's instructions.

IL-1 Hy2 partially inhibited IL-1β-stimulated IL-6 production in a dose-dependent manner. In view of the fact that IL-6 blocks production of tumor necrosis factor (TNF), a pro-inflammatory cytokine, the fact that IL-1 Hy2 only partially inhibits of IL-6 production by IL-1 Hy2 may be beneficial in the treatment of inflammatory disease states with IL-1Hy2 due to reduced side effects. It is possible that more highly purified preparations of IL-1 Hy2 may show complete inhibition in this assay.

EXAMPLE 11

Inhibition of IL-18 Activity by IL-1 Hy2

The following experiment evaluated the ability of IL-1 Hy2 to inhibit IL-18 activity, as measured by induction of IFN-γ. Human lymphocytes (PBMC) were obtained by Ficoll-Hypaque density gradient separation of peripheral blood from healthy volunteer donors. Immediately after isolation, the PBMC were washed two times with growth media, containing RPMI 1640-10% fetal bovine serum, and 3 x 10^5 cells/well were seeded in a 96 well plate. The cells were stimulated by adding anti-CD3 antibody (R & D Systems, Minneapolis, MN) to all of the samples at a final concentration of 0.5 µg/ml. At the time of stimulation, all but one control well per plate were treated with 100 ng/ml recombinant IL-18 (R&D Systems) for 36 hours at 37°C at 5% CO_2. The untreated well served as a measure of background levels of IFNγ produced by stimulated PBMC cells. IL-18 treatment causes the PBMC cells to increase production of IFN-γ relative to the background levels.

To assay for IL-1 Hy2 inhibition of IL-18 stimulated IFNγ production, 100x fold to 1000x fold concentration of IL-1 Hy2 (relative to IL-18 concentration) was added to wells together with IL-18 at the time of stimulation. After 36 hours of cell stimulation, the culture

plate was centrifuged for 5 minutes at 4000 rpm to remove cell debris. The supernatant was assayed for IFNγ using the Quantikine IFNγ ELISA kit (R & D Systems) according to the manufacturer's suggested protocol.

Results indicated that IL-18 alone stimulated IFNγ production and that IL-1 Hy2 had some inhibitory activities on the IL-18 stimulation. In order to assess the mechanism by which IL-1 Hy2 reduced IFNγ production, the following assay was carried out.

Human lymphocytes (PBMC) were obtained, washed, seeded, stimulated with anti-CD3 antibody and treated with a final concentration of 100 ng/ml IL-18 (R & D Systems) as described above. Several blocking antibodies were then used to test inhibition of IFNγ production, including anti-IL 18 receptor antibody, anti-IL-1 receptor accessory protein antibody, anti-IL1 receptor type I antibody and anti-IL-1 receptor type II antibody (all obtained from R & D Systems, Minneapolis, MN). Different amounts of each antibody were added to the wells with IL-18, and after 36 hours of cell stimulation, the culture plate was centrifuged for 5 minutes at 4000 rpm to remove cell debris. The supernatant was assayed for IFNγ using the Quantikine IFNγ ELISA kit (R & D Systems) according to manufacturer's instructions.

In the absence of an antibody, IL-18 stimulated IFNγ production relative to background levels as observed above. However, anti-IL18 receptor antibody, anti-accessory protein antibody and anti-IL-1 receptor type I, but not type II, antibody inhibited IL-18 induced IFNγ production.

These results indicate that compounds which antagonize the action of the IL-1 receptor inhibit IL-18 activity as measured by induction of IFNγ production.

EXAMPLE 12

Binding of IL-1 Hy2 to the Interleukin-1 Receptor

A cell binding assay was carried out, in a modification of the procedure as described above in Example 7.1, to determine if IL-1 Hy2 of the invention binds to the interleukin-1 (IL-1) receptor. Briefly, fluorescent activated cell sorting (FACS) was used to measure cell binding of the recombinant protein (see Example 6) using fluorescent antibodies specific for the express tag on the IL-1 Hy2 recombinant protein. In each reaction, 10^6 cells of human fibroblast cells (CCD 1089) were suspended in 100 µl of FACS

buffer (containing distilled PBS, 3% calf serum and 0.01% azide). Cell binding reactions included 5 nM recombinant IL-1 Hy2 in 100 µl cell suspension. The cells were incubated on ice for one hour. The cells were pelleted by centrifugation, 200 µl of 0.2 mM BS3 (crosslinker) was added, and the cells were kept on ice for 30 minutes. Next, 10 µl 1 M Tris pH 7.5 was added and the cells were incubated for 15 minutes on ice. The cells were pelleted by centrifugation, washed one time in FACS buffer, resuspended in 100 µl volume of FACS buffer, 2 µl primary antibody (anti-express tag antibody 1mg/ml) was added, and incubation continued on ice for an additional 30 minutes. The cells were pelleted by centrifugation, washed with FACS buffer, and resuspended in FACS buffer (100 µl volume). The secondary antibody (phycoerythrin-conjugated), 2 µl of anti-mouse Ig (1mg/ml), was added and the cells were incubated for 30 minutes on ice. The cells were again pelleted by centrifugation, washed two times with FACS buffer, resuspended in 0.5 ml FACS buffer and analyzed on FACS.

A shift in the fluorescence was observed for the cells treated with the recombinant tagged IL-1 Hy2. This binding was specific, as binding was not observed with the same molarity of non-related proteins, such as bovine serum albumin (BSA). Specific IL-1 Hy2 binding was also demonstrated in the murine T cell line D10 and the murine monoclonal cell line RAW 264.7. These results indicate binding of the IL-1 Hy2 protein of the invention to the IL-1 receptor.

EXAMPLE 13

Expression of IL-Hy2 Polypeptide in Mammalian Cells

To express IL-1 Hy2 in mammalian cells, Chinese hamster ovary (CHO) cells were transfected with a mammalian expression vector and IL-1 Hy2 secretion was detected. The protein coding region of IL-1 Hy2 was obtained by PCR. The IL-1 Hy2 cDNA was used as a template for the IL-1 Hy2 specific primers (5′ ATGGTTCCCTCCCCATGGCAAG3′ and 5′ GCTACCAGC TCTGTTCAAAGT AAAAC3′; SEQ ID NO: 19 and 20 respectively) designed to amplify the shorter ORF. The PCR reaction was run for 30 cycles at 94°C for 30 seconds, 55°C for 30 seconds and 72°C for 30 seconds. The PCR product was inserted into the pcDNA3.1/V5-His-Topo vector (Invitrogen) per manufacturer's instructions. The resulting expression construct was sequenced to confirm that the inserted IL-1 Hy2 sequence was correct.

CHO cells were transiently transfected with the mammalian expression vector, pcDNA IL-1 Hy2, using the FuGene transfection reagent (Roche Molecular Biochemicals) according to manufacturer's instructions. The culture medium was collected 48 hours after the transfection and passed through a 0.2 μ filter to remove cellular debris.

The collected conditioned medium was concentrated 10 fold using microcolumns (Amicon) according to the manufacturer's instructions and analyzed by electrophoresis on a 10% SDS-polyacrylamide gel followed by Western blot hybridization. IL-Hy2 was detected on the Western blot with a polyclonal antibody specific for IL-1 Hy2 using the BCIP/NBT (Sigma) according to the manufacturer's instructions. The polyclonal antibody used for the Western Blot was the IL-1 Hy2 specific antibody described in Example 3. The IL-1 Hy2 polypeptide was detected in both the cell culture medium and in the cell lysate, suggesting that IL-1 Hy2 is a secreted polypeptide of the apparent molecular weight 25 kD when expressed in mammalian cells. The IL-1 Hy2 polypeptide expressed in mammalian cells can be sequenced to confirm the amino terminus sequence of the mature protein.

The present invention is not to be limited in scope by the exemplified embodiments which are intended as illustrations of single aspects of the invention, and compositions and methods which are functionally equivalent are within the scope of the invention. Indeed, numerous modifications and variations in the practice of the invention are expected to occur to those skilled in the art upon consideration of the present preferred embodiments. Consequently, the only limitations which should be placed upon the scope of the invention are those which appear in the appended claims. All references cited within the body of the instant specification are hereby incorporated by reference in their entirety.

WHAT IS CLAIMED IS:

1. An isolated polynucleotide comprising a polynucleotide selected from the group consisting of:

(a) a polynucleotide having the nucleotide sequence of SEQ ID NO: 1, 12 or 14;

(b) a polynucleotide having the nucleotide sequence of the cDNA insert of clone pIL-1Hy2 (ATCC Accession No. PTA-96);

(c) a polynucleotide having the IL-1Hy2 protein coding nucleotide sequence of a polynucleotide of (a) or (b);

(d) a polynucleotide having the mature protein coding sequence of SEQ ID NOS: 1, 12 or 14.

2. An isolated polynucleotide encoding a polypeptide with IL-1 Hy2 activity, comprising a polynucleotide selected from the group consisting of:

(a) polynucleotides that encode the amino acid sequence of SEQ ID NO: 2;

(b) polynucleotides that encode the amino acid sequence of SEQ ID NO: 13 and

(c) polynucleotides that encode the protein encoded by the cDNA insert of clone pIL-1Hy2;

(d) polynucleotides that encode the mature amino acid sequence of SEQ ID NOS: 2 or 13.

3. An isolated polynucleotide encoding a polypeptide with IL-1 Hy2 activity that hybridizes under stringent conditions to the complement of a polynucleotide of any one of claims 1 or 2.

4. The polynucleotide of any one of claims 1 through 3 which is a DNA.

5. The polynucleotide of claim 3 which is an allelic variant.

6. The polynucleotide of claim 5 which is selected from the group consisting of polynucleotides having the IL-1Hy2 protein coding sequence of SEQ ID NO: 1 and comprising one or more of the following nucleotide changes: T125C, C184T and A205C.

7. An isolated polynucleotide which comprises a complement of the polynucleotide of Claim 1.

8. An expression vector comprising the DNA of Claim 4.

9. A host cell genetically engineered to contain the DNA of Claim 4.

10. A host cell genetically engineered to contain the DNA of Claim 4 in operative association with a regulatory sequence that controls expression of the DNA in the host cell.

11. An isolated polypeptide with IL-1 Hy2 activity comprising:
(a) the IL-1 Hy2 protein sequence of SEQ ID NOS: 2 or 13; or
(b) an amino acid sequence encoded by the cDNA insert of clone pIL-1Hy2 (ATCC Accession No. PTA-96); or
(c) a mature protein sequence of SEQ ID NOS: 2 or 13; or
(d) a polypeptide encoded by the polynucleotide of claim 3; or
(e) a polypeptide having an amino acid sequence 90% identical to the sequence of (a), (b), or (c).

12. A composition comprising the polypeptide of Claim 11 and a carrier.

13. An antibody directed against the polypeptide of Claim 11.

14. A method for detecting a polynucleotide of Claim 1 in a sample, comprising the steps of:

a) contacting the sample with a compound that binds to and forms a complex with the polynucleotide for a period sufficient to form the complex; and

b) detecting the complex, so that if a complex is detected, a polynucleotide of Claim 1 is detected.

15. A method for detecting a polynucleotide of Claim 1 in a sample, comprising the steps of:

a) contacting the sample under stringent hybridization conditions with nucleic acid primers that anneal to a polynucleotide of Claim 1 under such conditions; and

b) amplifying the polynucleotides of Claim 1 so that if a polynucleotide is amplified, a polynucleotide of Claim 1 is detected.

16. The method of Claim 15, wherein the polynucleotide is an RNA molecule that encodes a polypeptide of Claim 11, and the method further comprises reverse transcribing an annealed RNA molecule into a cDNA polynucleotide.

17. A method for detecting a polypeptide of Claim 11 in a sample, comprising:

a) contacting the sample with a compound that binds to and forms a complex with the polypeptide for a period sufficient to form the complex; and

b) detecting the complex, so that if a complex is detected, a polypeptide of Claim 11 is detected.

18. A method for identifying a compound that binds to a polypeptide of Claim 11, comprising:

a) contacting a compound with a polypeptide of Claim 11 for a time sufficient to form a polypeptide/compound complex; and

b) detecting the complex, so that if a polypeptide/compound complex is detected, a compound that binds to a polypeptide of Claim 11 is identified.

19. A method for identifying a compound that binds to a polypeptide of Claim 11, comprising:

a) contacting a compound with a polypeptide of Claim 11, in a cell, for a time sufficient to form a polypeptide/compound complex, wherein the complex drives expression of a reporter gene sequence in the cell; and

b) detecting the complex by detecting reporter gene sequence expression, so that if a polypeptide/compound complex is detected, a compound that binds to a polypeptide of Claim 11 is identified.

20. A method of producing the polypeptide of Claim 11, comprising,

a) culturing the host cell of claim 9 for a period of time sufficient to express the polypeptide contained within said cell; and

b) isolating the polypeptide from the cell of step 1.

21. A polypeptide according to Claim 11 capable of binding to Interleukin-1 Receptor.

22. A polypeptide according to Claim 11 which is an antagonist of Interleukin-1 Receptor.

23. A method of modulating the biological activity of an Interleukin-1 Receptor comprising contacting the receptor with the polypeptide of Claim 11.

24. The polypeptide of Claim 11 coupled to a label.

25. A method of labeling an Interleukin-1 (IL-1) receptor comprising contacting said IL-1 receptor with a labeled polypeptide of claim 24.

26. A kit comprising the polypeptide of Claim 11.

27. A collection of polynucleotides, the collection comprising the sequence information of at least one of SEQ ID NOS: 1, 12 or 14 or fragments thereof.

28. The collection of claim 27, wherein the collection is provided on a nucleic acid array.

29. The collection of claim 27, wherein the collection is provided in a computer-readable format.

30. A method of treatment for cancer involving elevated levels of IL-1 comprising the step of:

administering to a patient in need thereof an amount of IL-1 Hy2 effective to ameliorate symptoms of said cancer.

31. The method of claim 30 wherein the cancer is selected from the group consisting of breast adenocarcinoma, brain tumors, melanoma, myeloma, giant cell tumors of bone, acute myelogenous leukemia, oral epidermoid carcinoma and squamous cell carcinoma.

32. A method of treating an inflammatory disease state mediated by IL-18 comprising administering to a subject in need thereof an amount of an IL-1 Hy2 polypeptide of claim 11 effective to inhibit IL-18 activity.

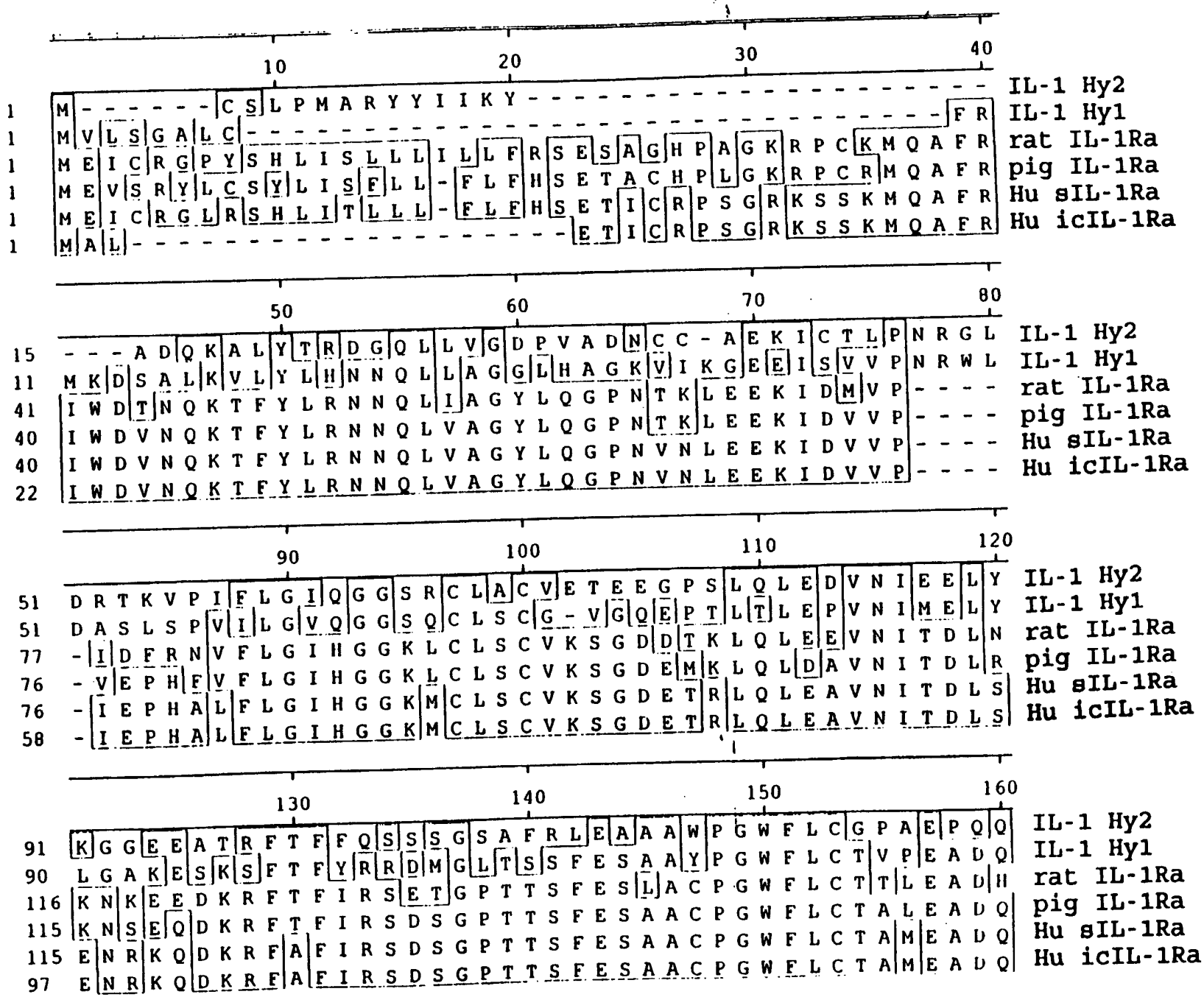
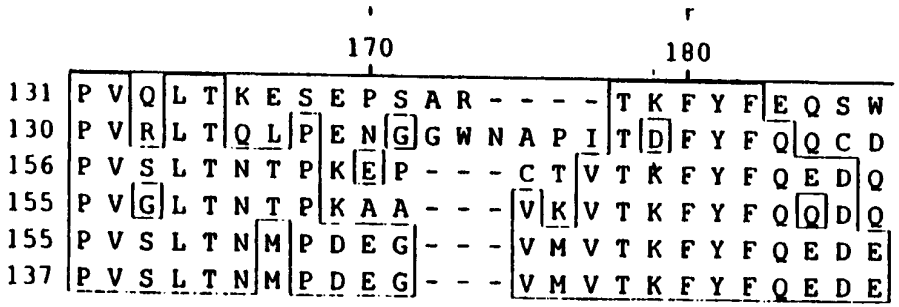


FIG.1A



IL-1 Hy2
IL-1 Hy1
rat IL-1Ra
pig IL-1Ra
Hu sIL-1Ra
Hu icIL-1Ra

Decoration 'id-consensus': Box residues that match the Consensus exactly.

FIG.1B

FIGURE 2

SEQ ID NO.: 12

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ATACACAAGA GACGGCCAGC TGCTGGTGGG AGATCCTCTT GCAGACAACT GCTGTGCAGA 540
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FIGURE 3

SEQ ID NO.: 13

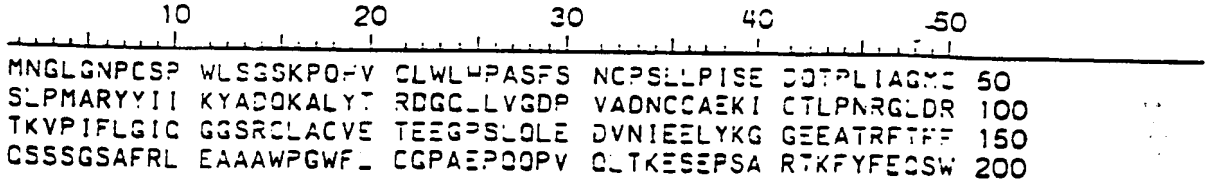

FIGURE 4

SEQ ID NO.: 14

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CTGGAAGCCT TGGTGCATTC TGGCAGGCCA ATTATAGACG AATCGCCTGG GGAACCCGTG 300
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Gln Lys Ala Leu Tyr Thr Arg Asp Gly Gln Leu Leu Val Gly Asp Pro
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<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

<400> 20

gctaccagct ctgttcaaag taaaac 26

INTERNATIONAL SEARCH REPORT

Int. Ional Application No
PCT/US 00/14144

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/25 C07K14/54 C12Q1/68 C07K14/545 C07K16/24
C12N15/24 G01N33/68 A61K38/20

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, STRAND, BIOSIS, CHEM ABS Data, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 98 47921 A (SCHERING CORP) 29 October 1998 (1998-10-29) claims Sequence ID no.2 page 90	1-32
A	CARTER D B ET AL: "PURIFICATION, CLONING, EXPRESSION AND BIOLOGICAL CHARACTERIZATION OD AN INTERLEUKIN-1 RECEPTOR ANTAGONIST PROTEIN" NATURE,GB,MACMILLAN JOURNALS LTD. LONDON, vol. 344, no. 6267, 12 April 1990 (1990-04-12), pages 633-638, XP002038695 ISSN: 0028-0836 the whole document	1-32

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 October 2000	18/10/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Le Cornec, N

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INTERNATIONAL SEARCH REPORT

Int: ional Application No
PCT/US 00/14144

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,A	DATABASE EMBL 'Online! AQ766579, accession number AQ766579, 30 July 1999 (1999-07-30) G.G. MAHAIRAS ET AL: "Sequence tagged connectors: a sequence approach to mapping and scanning the human genome" XP002149452 abstract * 85,52% identity in 304 nt overlap with sequence ID no.1, no.12 and no.14 * & PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA., vol. 96, no. 17, 1999, pages 9739-9744, WASHINGTON US	1-10
P,A	WO 99 51744 A (HYSEQ INC) 14 October 1999 (1999-10-14) the whole document	1-32
P,A	DATABASE EMBL 'Online! AC016724, accession number AC016724, 14 December 1999 (1999-12-14) R.H. WATERSTON : "The sequence of Homo sapiens clone. Homo sapiens chromosome 2 clone RP11-339F22" XP002149453 * 99,28% identity in 699 nt overlap (666-1364:121481-122179) with seq ID no.12 and no.14 * abstract & UNPUBLISHED,	1-10

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INTERNATIONAL SEARCH REPORT

Int ional Application No
PCT/US 00/14144

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9847921 A	29-10-1998	AU 7103198 A	13-11-1998
		EP 0977779 A	09-02-2000
WO 9951744 A	14-10-1999	AU 3449599 A	25-10-1999